Incidence of Fatal or Repaired Abdominal Aortic Aneurysm in Relation to Inflammation-Sensitive Plasma Proteins

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Background—Inflammation is an important pathophysiological feature of abdominal aortic aneurysms (AAA). Whether elevated levels of inflammation-sensitive plasma proteins (ISPs) predict the long-term risk of fatal or repaired AAA is largely unknown.

Methods and Results—Five ISPs (fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin, and ceruloplasmin) were measured in 6075 healthy men, mean age 46.8±3.7 years. After a mean time of 19 years, 63 men had a fatal or surgically/endothelially repaired AAA. Risk of treatment or death from future AAA was studied in relation to the ISPs. The risk of future AAA increased significantly with the number of elevated ISPs (ie, in the top quartile). The proportions with future AAA were 0.4%, 1.0%, 1.3%, and 2.3% for men with none, one, two, and ≥3 ISPs, respectively, in the top quartile (trend: \( P<0.0001 \)). The corresponding odds ratios were 1.00 (reference), 1.9 (95% CI: 0.8 to 4.5), 2.2 (0.9 to 5.5), and 3.2 (1.4 to 7.2), respectively, adjusted for age, screening year, smoking, cholesterol, triglycerides, systolic blood pressure and physical inactivity (trend: \( P=0.004 \)).

Conclusion—The incidence of fatal or repaired AAA is associated with the ISP levels. In this population-based study, elevated ISPs could be observed many years before the clinical manifestation of disease. (Arterioscler Thromb Vasc Biol. 2004;24:337-341.)

Key Words: aneurysm ■ inflammation ■ epidemiology

Inflammation with infiltrates of macrophages and lymphocytes is an important feature of abdominal aortic aneurysms (AAA). Elevated levels of various plasma markers of inflammation have been reported in patients with AAA, as compared with healthy controls or patients with cardiovascular disease. Positive correlations between inflammatory markers and the degree of aortic dilatation have been reported in cross-sectional studies. Based on these findings and results from experimental studies, it has been suggested that inflammation occurs early in the development of AAA and that inflammation has a causal role. However, whether plasma markers of inflammation predict future AAA in long-term prospective studies is largely unknown.

The cohort “Malmö Preventive Study” includes 6075 healthy men with information on 5 inflammation-sensitive plasma proteins (ISPs; fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin, and ceruloplasmin). We have previously shown that elevated levels of these inflammatory markers were associated with an increased incidence of stroke and myocardial infarction over an 18-year follow-up period. This study explored whether elevated ISP levels are associated with an increased risk of developing a fatal or repaired AAA.

Methods
Between 1974 and 1984, 22,444 men participated in a screening program for detection of individuals with high risk for cardiovascular diseases. The men were 27 to 61 years old at screening. Participation rate was 71%. Determination of the 5 plasma proteins was part of the program for 6193 men, mainly from the older age-groups, who were examined between 1974 and 1982. After exclusion of men with a history of myocardial infarction, stroke, or cancer (according to questionnaire), 6075 men remained, with a mean age of 46.8±3.7 years (range: 28 to 61). The health service authority of Malmö approved the screening program. All participants gave informed consent.

In order to evaluate the representativity of the subcohort with data on ISPs, this group was compared with remaining cohort that belonged to the same age groups and was screened during the same period. After adjustments for age, there was no difference in mean systolic blood pressure (128.4 versus 128.4 mm Hg), cholesterol (5.7 versus 5.7 mmol/L), or prevalence of diabetes (5.4 versus 5.7%). Prevalence of smoking was slightly lower in men with data on ISPs (48 versus 50%).

Baseline Examinations
Subjects were categorized into nonsmokers and smokers. Tobacco consumption was divided into daily consumption of less than 10 cigarettes, 10 to 19 cigarettes, and 20 cigarettes or more.

Blood pressure (mm Hg) was measured twice in the right arm after a 10-minute rest, and the average of the two measurements was used. A sphygmomanometer and a rubber cuff of appropriate size were

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used. Use of anti-hypertensive medication was assessed in a questionnaire.

Blood samples were taken after an overnight fast and analyzed at the Department of Clinical Chemistry at Malmö University Hospital. Serum cholesterol and triglyceride concentrations were analyzed with standard methods.

Physical activity was assessed in a questionnaire. Two categories of physical activity were used, sedentary or not.

Men with fasting whole blood glucose ≥6.1 mmol/L, and/or 2-hour glucose ≥10.0 mmol/L, and men who reported that they had diabetes, were considered diabetic.14

BMI was calculated as weight/height² (kg/m²).

Inflammation-Sensitive Plasma Proteins (ISPs)

Electroimmuno assay was used to analyze the plasma levels of 5 ISPs.15 The analysis was performed consecutively at the time of screening. The precision of the analysis had an error below 5%.15 The detection limits were 20 mg/L for ceruloplasmin, 50 mg/L for α1-antitrypsin, and 350 mg/L for orosomucoid, haptoglobin, and fibrinogen.

We have previously shown that the correlation coefficients between the individual proteins range between 0.31 to 0.56 and that the cardiovascular risk increases with the number of ISPs in the top quartile.12,14,16,17 In accordance with the previous studies, the sample was therefore categorized according to the number of elevated ISPs.

Patients

The Swedish Vascular registry (SWEDVASC)18 and the local and national inpatient registers were used to identify patients treated for AAA with an open surgical or endovascular procedure at the Malmö University Hospital or the Lund University Hospital. The uptake area of these hospitals covers the population of Malmö and the surrounding cities and areas. Fatal cases of AAA (ICD-9 code 441, ICD-10 code I71) were retrieved from the Swedish Causes of Death Register. For cases retrieved from sources other than the SWEDVASC registry, the diagnosis was confirmed by review of autopsy protocols and/or hospital records. Of the 13 fatal cases, cause of death was based on autopsy results in 9 cases (70%), and on examinations in-hospital before death in 4 cases.

Statistics

One-way ANOVA and Pearson’s χ² were used to compare risk factors among patients and controls. Logistic regression was used to study the relationships between ISPs and AAA with adjustments for potential confounders. A general linear model was used to adjust the relation between ISPs and time to event for age and screening year.

Results

A total of 63 men had AAA (0.49 per 1000 person years). Fifty were nonfatal cases whose aneurysm was repaired in an open vascular or endovascular surgical procedure, and 13 of those 50 (26%) were ruptured. The remaining 13 cases were fatal. The mean time from the baseline examination to aneurysm repair or death from AAA was 18.8±4.9 years (range 1.3 to 26.6). Age at the time of the AAA was 67.1±5.3 years (range: 55 to 80 years).

Baseline Characteristics of Men with AAA and Controls

The number of elevated ISPs was significantly higher in men who subsequently had AAA as compared with the controls (Table 1). The numbers with future AAA were 9 (0.4%), 15 (1.0%), 12 (1.3%), and 27 (2.3%), respectively, for men with 0, 1, 2, and ≥3 ISPs in the top quartile (P for trend <0.0001). Similar relationships were observed for all individual ISPs. Prevalences of smoking, mean age, triglycerides, and choles-

terol at baseline were also significantly higher among men with future AAA. There was no significant difference at baseline between men who later had nonruptured AAAs (n=37) and men with ruptured or fatal AAA (n=26) (Table 1).

In men who were below the median age (47.4 years) at the screening examination, the numbers with future AAA were 2 (0.2%), 6 (0.8%), 2 (0.5%), and 8 (1.3%), respectively, for men with 0, 1, 2, and ≥3 ISPs in the top quartile (P for trend=0.006). The corresponding figure for men above the median age were 7 (0.6%), 9 (1.1%), 10 (2.1%), and 19 (3.4%), respectively (P for trend <0.0001). In both age-groups, the associations remained significant after adjustments for age and screening year.

Multivariate Analysis

Table 2 presents the adjusted odds ratios comparing the number of elevated ISPs in men with future AAA and controls. The odds of AAA increased with the number of elevated ISPs. This relationship remained statistically significant after adjustments for potential confounders. The relationships were largely similar for nonruptured and ruptured/fatal AAAs (Table 2).

All individual ISPs were significantly associated with future AAA after adjustments for age and screening year. After adjustments for potential confounders, ceruloplasmin and fibrinogen remained significant (Table 3).

ISPs in Relation to Time to AAA

The time period from the baseline examination to the AAA was 22.5±3.4, 18.2±6.2, 19.7±4.4, and 17.6±4.9 years, respectively, for cases with 0, 1, 2, and ≥3 ISPs in the top quartile (P for trend=0.03). However, this relation was no longer significant after adjustment for age and screening year (P=0.10). The risk factor-adjusted relationships between number of elevated ISPs and AAA was still significant when men who had AAA during the first 10 years of follow-up (n=4) were excluded.

Discussion

Inflammation is a prominent feature of AAAs and several studies have reported associations between AAA and various inflammatory markers.1–11 However, whether elevated ISPs are predictive for AAA in a long-term prospective study is largely unknown. The present results show that elevated ISPs in apparently healthy men are associated with an increased risk of developing AAA. Elevated levels of these inflammatory markers could be observed many years before the clinical manifestation of disease.

Prospective studies of the relationships between inflammation and incidence of AAA are scarce. A nested case-control study reported higher fibrinogen levels in cases as compared with controls.5 Because of the relatively short follow-up period, it was suggested that the increased fibrinogen concentrations were explained by fibrin deposition in pre-existing aneurysmal sacs. Cross-sectional studies have reported associations between AAA and fibrinogen, C-reactive protein (CRP), and other inflammatory markers.6–9 To our
knowledge, there are no long-term prospective studies on incidence of AAA in relation to these proteins.

Inflammation has an important role in the development of atherosclerosis. Many prospective studies have reported associations between various markers of inflammation and atherosclerotic diseases, including myocardial infarction, stroke, peripheral vascular disease, and carotid atherosclerosis. However, although AAA is strongly associated with atherosclerosis, the development of AAA is also a result of a proteolytic degradation and remodelling of the elastic

| TABLE 1. Risk Factors at Baseline for Men With and Without a Fatal or Repaired AAA |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                 | All AAA                         | Nonruptured                    | Rupture/death                   | Controls                        | P (all AAA vs controls)        |
| Age at baseline (years)         | 48.4±3.9                       | 47.7±2.5                      | 49.2±5.2†                      | 46.8±3.7                       | 0.001                          |
| Time to AAA (years)             | 18.8±4.9                       | 18.9±4.3                      | 18.8±5.8                       |                                 |                                |
| Myocardial infarction after the screening, n (per 1000 person-years) | 10 (8.74)                      | 4 (5.61)                      | 6 (13.9)                       | 603 (5.50)                     | 0.16                           |
| SBP (mm Hg)                     | 131±18                         | 128±15                        | 134±22                         | 129±16                         | 0.29                           |
| DBP (mm Hg)                     | 88±11                          | 89±10                         | 88±13                          | 87±10                          | 0.39                           |
| Anti-hypertensive medication (%) | 6.3                            | 2.7                           | 11.5                           | 4.6                            | 0.51                           |
| BMI (kg/m²)                     | 25.3±3.3                       | 24.9±2.8                      | 25.8±3.9                       | 25.0±2.7                       | 0.44                           |
| Cholesterol (mmol/L)            | 6.3±1.0                        | 6.2±1.0†                      | 6.3±1.1†                       | 5.7±1.0                        | <0.001                         |
| Triglycerides (mmol/L)          | 1.9±1.2                        | 1.9±1.2†                      | 1.8±1.2                        | 1.6±1.1                        | 0.03                           |
| Smokers (%)                     | 18                             | 29                            | 18                             | 25                             | 0.46                           |
| Angina (%)                      | 1.6                            | 2.7                           | 0                              | 1.3                            | 0.84                           |
| Diabetes (%)                    | 1.6                            | 0                             | 3.8                            | 5.5                            | 0.18                           |
| Physical inactivity (%)         | 67                             | 62                            | 73                             | 56                             | 0.10                           |
| α1-antitrypsin (g/L)            | 1.40±0.28                      | 1.42±0.29†                    | 1.38±0.28†                     | 1.27±0.27                      | <0.001                         |
| Ceruloplasmin (g/L)             | 0.36±0.07                      | 0.35±0.07†                    | 0.36±0.09†                     | 0.32±0.07                      | <0.001                         |
| Fibrinogen (g/L)                | 3.95±0.65                      | 4.00±0.73†                    | 3.90±0.53†                     | 3.50±0.80                      | <0.001                         |
| Haptoglobin (g/L)               | 1.69±0.79                      | 1.83±0.80†                    | 1.50±0.75†                     | 1.38±0.68                      | <0.001                         |
| Orosomucoid (g/L)               | 0.91±0.23                      | 0.94±0.25†                    | 0.87±0.19                      | 0.82±0.20                      | <0.001                         |
| No. of ISP in the top quartile  |                                |                               |                                |                                |                                |
| None (%)                        | 14                             | 19                            | 7.7                            | 41                             | Trend: <0.001                   |
| One (%)                         | 24                             | 16                            | 35                             | 26                             |                                |
| Two (%)                         | 19                             | 16                            | 23                             | 15                             |                                |
| Three or more (%)               | 43                             | 49†                           | 35†                            | 19                             |                                |
| ISP in top quartile, n          | 2.3±1.6                        | 2.4±1.7†                      | 2.2±1.5†                       | 1.2±1.4                        | <0.0001                        |

Values are mean±SD, unless otherwise stated.
†significantly different vs controls.

| TABLE 2. Adjusted Odds Ratios Comparing Risk of Fatal or Repaired AAA in Relation to No. of ISP in the Top Quartile |
|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|
| ISPs in the Top Quartile                                     | None              | One               | Two               | Three or more               | P for trend                       |
| All AAA                                                      | Model 1            | 1.00              | 2.6 (1.1–6.0)            | 3.6 (1.5–8.6)            | 6.4 (3.0–14)            | <0.0001                       |
| Model 2                                                      | 1.00               | 1.9 (0.8–4.4)            | 2.2 (0.9–5.4)            | 3.2 (1.4–7.1)            | 0.008                         |
| Non-ruptured AAA                                            | Model 1            | 1.00               | 1.4 (0.5–4.1)           | 2.4 (0.8–7.0)           | 5.8 (2.4–14)           | 0.0001                        |
| Rupture/death                                               | Model 1            | 1.00               | 6.9 (1.4–32)            | 7.9 (1.6–39)            | 8.8 (1.9–41)            | 0.004                         |

Model 1: Adjusted for age at baseline and screening year.
Model 2: Model 1 + smoking, cholesterol, triglycerides, systolic blood pressure, and physical inactivity (due to limited No. of cases, model 2 was only applied for "All AAA").
extent. It has been reported that the elastolytic activity in the arterial wall is associated with inflammation. The plasma level of the proteolytic agent matrix metalloproteinase (MMP)-9 is associated with fibrinogen and CRP among patients with cardiovascular disease. However, it is unclear whether an increased development of atherosclerosis, an increased degradation of the elastic media, or both, explains the relationships between ISPs and incidence of AAA in initially healthy men. In this study the ISP levels were significantly increased at a mean time of 19 years before the clinical manifestation of the disease. This suggests that elevated ISPs also may reflect a predisposition to react with increased inflammatory responses, rather than ongoing inflammatory processes in the vessel wall. AAAs tend to cluster within families, and there seems to be a genetic component for the development of AAA. It has also been shown that, eg, the fibrinogen level is genetically determined to a large extent.

In most screening situations the incidences of the diseases are low and, as a consequence, the positive predictive values are low. Measurement of inflammatory markers for the prediction of treatment or death from AAA is no exception. In this study, three elevated ISPs correspond to a sensitivity of 43%. Because no examinations were performed to exclude AAA in control subjects with low ISPs, the specificity is unclear. If all men with low ISPs in the control group were without AAA, the specificity would be 81%. Because of the low incidence, the positive predictive value would be only 2.4%. However, the blood tests were performed in relatively young men, both smokers and nonsmokers, many years before the treatment or death from AAA. Even though the predictive value for future AAA was low in this setting, it should be higher in specific high-risk groups. As elevated ISPs are strongly associated with myocardial infarction and stroke, two diseases with higher incidences, it is still likely that a panel of inflammatory markers can be useful for the identification of individuals with high cardiovascular risk.

Modern ultrasound techniques to assess the diameter of the abdominal aorta were not available when the baseline examination was performed, and no specific examination was performed to diagnose asymptomatic AAA at baseline. As the temporal course of the development of aneurysms is poorly known, we cannot completely rule out the possibility that the development of AAA already could have started in some men, and that this could have increased the ISPs. However, there was a long time between the baseline examination and the AAA events, and the relationships remained after exclusion of events during the first 10 years of follow-up. Another question is whether some patients with AAA were more likely to be scheduled for elective surgery, and whether such bias could be related to baseline ISP levels. However, as the relations were similar for nonruptured and ruptured/fatal AAAs, it is very unlikely that this relation explains the results.

We do not know whether the growth rate of the aneurysms was associated with the ISPs. A previous study reported correlations between interferon-γ and the expansion rate, while another study showed no association between IL-6 and growth rate. Although not significant, there was an inverse relationship between the number of elevated ISPs and the time to the AAA in the present study, which could indicate that the growth rate is higher in men with high ISPs.

It is concluded that the incidence of fatal or repaired AAA is significantly associated with the ISP levels. In this population-based study, elevated ISPs could be observed many years before the clinical manifestation of disease.

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